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Reduced ethylene production in tomato fruits upon CRISPR/Cas9-mediated LeMADS-RIN mutagenesis

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Recent progress in genome editing methods has opened new opportunities for reverse genetics-based studies in plants. The clustered regularly interspaced short palindromic repeat (CRISPR) system is a novel strategy used to induce mutations in a specific genomic region of a variety of organisms, including plants. Here, we describe a high-frequency targeted mutagenesis utilizing *Agrobacterium*-delivered CRISPR/Cas9 in tomato. This system consists of an *Agrobacterium* binary vector and three guide RNAs for single gene targeting. We evaluated the system for its mutagenesis frequency and heritability using LeMADS-RIN gene of tomato. T_0 transgenic events carrying mutations in the LeMADS-RIN gene occurred at rates over 10.6 % mutants per transgenic event in both 'Mamirio' and 'Golden bell' tomato genotypes. Three independent T_1 transgenic lines and wild-type (WT) tomato plants were used for ethylene analysis. Compared with WT plants, edited mutants exhibited more incompletely-ripening fruits and lower ethylene contents. Following genetic combination through segregation, null segregants carrying only the desired mutant alleles without the CRISPR transgene could be retrieved among the T_1 progeny. These Cas9/gRNA transgenic lines, therefore, can be used to convey the CRISPR-based mutagenesis by genetic cross to tomato lines that are not amenable to genetic transformation.

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